The Rickettsial Diseases of Man

Carl E. Pedersen, Jr.

[1.] *General Properties*

The rickettsial diseases endemic to North America include:

(1) Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*), (2) Q fever (caused by *Coxiella burnetii*), (3) endemic typhus (caused by *Rickettsia typhi*), (4) Rickettsialpox (caused by *Rickettsia akari*), and (5) Brill-Zinsser disease (caused by *Rickettsia prowazekii*). In addition there are several other rickettsial diseases which occur throughout the world. The study of rickettsiae has been emphasized in only a few laboratories in recent years; however, their potential for explosive epidemic outbreaks, coupled with the continually rising number of cases of Rocky Mountain spotted fever in this country, has spurred renewed interest in these agents. The clinical outcome of disease very often depends on correct early diagnosis and appropriate, timely therapy. This report is an attempt to familiarize the reader with the rickettsial diseases of man and the salient features which might be used for practical identification of the causative microorganisms.

Morphologically, rickettsiae resemble small, non-motile coccobacillary, bacillary or pleomorphic bacteria. The smallest *(C. burnetii)* measures 0.25 by 1.0 μm, while the largest (members of the spotted fever group) average 0.6 by 1.2 μm. They may be found singly, in pairs or chains. Rickettsiae stain poorly by Gram's method *(C. burnetii* appears Gram-positive; others Gram-negative); therefore, the staining technique developed by Gimenez (1964) is strongly recommended. Using this procedure rickettsiae appear red against a contrasting blue background. Bipolar staining can be observed. They share several characteristics with bacteria including: (1) division by binary fission; (2) contain DNA and RNA; (3) contain muramic and diaminopimelic acids in the mucopeptides of their complex cell walls; (4) possess enzymes for the Krebs cycle, electron transport system and protein synthesis; and (5) growth is inhibited by several antibacterial compounds. Rickettsiae are highly fastidious microbes which are obligate intracellular parasites (except *Rochalimaea quintana*). By appropriate staining all rickettsiae are observed intracytoplasmically and occasionally in the nucleus of cells (spotted fever group).

Diseases caused by rickettsiae vary from benign, self-limited illness, to some of the most fulminating known. Fatal-

ity rates range from less than 1% to as high as 90% in some untreated cases. Important differences in clinical manifestations and severity of disease are in part dependent upon the distribution of organisms and the extent of vascular involvement. The majority of rickettsial diseases are zoonoses with the primary cycle of infection in animal hosts. The outstanding exception is epidemic typhus, in which man is the reservoir. In nature rickettsiae are generally transmitted to man by arthropod vectors including ticks, lice, mites, and fleas. *C. burnetii* is the exception as it is disseminated primarily by aerosol. After a variable incubation period ranging from 1 to 4 weeks, the onset of rickettsial illness is generally abrupt, with severe headache, chills and fever, followed by a hemorrhagic rash. Once within the skin rickettsiae multiply within cells (division time is about 8 hours), circulate within the circulatory system (rickettsemia), and reach endothelial cells of small blood vessels. The infected cells enlarge, degenerate and cause thrombi. The vascular lumen becomes occluded, and with infiltration of leukocytes and plasma cells, focal necrosis ensues. This, in turn, leads to increased permeability, rupture, petechiae, hemorrhage, and shock. In the case of *C. burnetii* primary atypical pneumonia is the predominant lesion.

All rickettsial infections respond dramatically to adequate treatment with the broad spectrum tetracyclines (especially doxycycline) or chloramphenicol, supplemented with intensive supportive measures. These drugs are rickettsiostatic in action, not rickettsiocidal.

While rickettsiae contain the necessary metabolic equipment for survival, their membranes are permeable to essential components which may either "leak out" or be borrowed from the host cell. This may explain why these organisms have lost the ability to be self sufficient outside of host cells. These intracellular parasites are, therefore, protected from extracellular exposure by direct transmission from (1) arthropod to arthropod via infected ova, (2) mammal to arthropod via infected blood, and (3) arthropod to mammal via direct inoculation (bite). While most rickettsiae are fragile and readily inactivated outside of the intracellular environment, *C. burnetii* is extremely stable, resists degradation, and can be transmitted by the aerosol route.

Most rickettsiae, except *R. quintana*, grow luxuriantly in

the yolk sac of the developing chick embryo 6 or 7 days of age. After a variable incubation period at 35 C, smears show intracellular organisms. Suspensions of rickettsiae may be prepared from these yolk sacs by the method of Craigie, which involves formalin inactivation followed by ether extraction. Vaccines have traditionally been prepared from rickettsiae propagated in the chick embryo (Cox, 1941) or the intestinal tract of lice (Weigl, 1919). More recently, cell cultures have been used to propagate rickettsiae for use in vaccines or the production of antigens. In addition, cell culture techniques have been used for the primary isolation of spotted fever rickettsiae from ticks and other infected specimens, and spotted fever group rickettsiae can be quantitated by plaque assay.

Many laboratory animals may be infected experimentally with rickettsiae; however, the guinea pig and white mouse are employed extensively for primary isolation and identification. When attempting to isolate rickettsiae from man, blood (obtained prior to initiation of antibiotic therapy) is allowed to clot; serum is removed (and retained for serologic assay for antibodies), and the clot is ground as a 10% suspension in Brain Heart Infusion. This suspension is injected intraperitoneally into test animals. Guinea pigs may demonstrate several types of response involving fever, peritoneal exudate, inflammation of the tunica vaginalis with swelling and necrosis of the scrotum (Neill-Mooser reaction) and death. Spleen, brain and tunica vaginalis are tissues of choice for serial passage of rickettsiae. There is considerable variation in the pathogenicity of rickettsial species and strains within species.

Concentrated suspensions of typhus and spotted fever group rickettsiae as well as the Gilliam strain of R. tsutsugamushi kill mice within a few hours after intravenous inoculation. This "toxic effect" cannot be prevented by antirickettsial drugs, and ultraviolet irradiation diminishes infectivity without reducing toxicity. Death is caused by gross damage to endothelial cells with concomitant leakage of plasma, decrease in blood volume and shock. It has not been possible to separate a toxic component from the infectious particle, but the "toxin" is specific for the species of rickettsiae. The "toxic effect" is prevented by addition of homologous antiserum. Suspensions of some rickettsiae also contain hemolysins capable of lysing the red blood cells of chicks, rabbits, and man. The hemolysin is distinct from the toxin.

Table 1. Spotted Fever Group Rickettsiae Pathogenic for Man.

Etiologic Agent	Disease	Geographic Distribution
Subgroup A		
R. rickettsii	RMSF, Sao Paulo typhus, Fiebre manchada	Western Hemisphere
R. sibirica	Siberian tick typhus	Eastern Hemisphere
Subgroup B		
R. conorii	Fievere boutonneuse, South African tick bite fever, Kenya tick typhus, Indian tick typhus	Eastern Hemisphere
Subgroup C		
R. australis	Queensland tick typhus	Eastern Hemisphere
R. akari	Rickettsialpox	Northeast, USA, USSR

[2.] The Spotted Fever Group

The eight species in this group pathogenic for man include the etiologic agent of Rocky Mountain spotted fever (RMSF), the tick-borne rickettsiae of the Eastern Hemisphere and Rickettsialpox. All of these organisms are antigenically related in that they possess a common, soluble, group specific antigen, multiply in both the cytoplasm and nucleus of infected cells, and are transmitted to man by ticks and mites. The diseases caused by these rickettsiae have a worldwide distribution.

[A.] R. rickettsii causes RMSF in man after transmission via the bites of several species of infected Ixodid ticks; principally Dermacentor variabilis in the eastern United States and D. andersoni in the western United States. The disease was originally seen in parts of Montana and was first described in Idaho by Maxey at the turn of the century. Spotted fever has now been recognized in almost every state, and in parts of Canada, Mexico, Central and South America. Currently, RMSF is most prevalent in the South Atlantic states and it appears that in 1977 for the first time there will be over 1,000 reported cases.* In the early 1900's Ricketts experimentally transmitted the disease from man to guinea pigs and monkeys, and also transferred the disease from animal to animal by the wood tick. In 1919 Wolbach demonstrated the intranuclear multiplication of these rickettsiae. In 1941 Cox developed a vaccine prepared from rickettsiae propagated in the yolk sacs of chicken embryos.

Disease in man generally occurs 1 to 2 weeks after the bite of an infected tick. The hallmark symptoms include abrupt onset, severe frontal headache, fever, and rash which first appears on the extremities on about the 4th day of fever. This rash spreads centripetally toward the trunk whereas the reverse is characteristic of the typhus fevers. Rickettsemia can be demonstrated during the febrile period. Organisms are found in the endothelial cells of the vascular system in great number. Thrombocytopenia may occur and intravascular thrombosis may lead to necrosis of the skin and soft tissues. Marked azotemia is an unfavorable sign and frank renal failure or shock may occur in the severely ill patient. In mild or moderate cases, disease is usually terminated within two weeks and convalescence is rapid without sequelae; however, complications may be seen. In fatal cases death usually occurs during the latter part of the second week.

If the disease does not become far advanced it usually reponds dramatically to chloramphenicol or the tetracyclines and defervescence occurs in 3 to 4 days. Headache and toxic signs abate within 24 to 48 hours following drug administration and rash fades in 2 to 3 days. Appropriate supportive therapy must be instituted for symptomatic relief and to correct fluid electrolyte imbalances. Mortality rates are low (ca. 5%) when the disease is recognized early and treated promptly. Without appropriate antibiotic therapy the case fatality rate approaches 20%.

Differential diagnosis is predicated primarily on clinical observation and serologic tests. The rash of RMSF must be distinguished from meningococcemia, rubella, rubeola, varicella and typhoid fever. History of tick-bite is very helpful. The Weil-Felix reaction is positive, but variable (Table 2).

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* In 1977, 1,115 cases were reported by the CDC.

Rickettsial Diseases

Table 2. *Weil-Felix Reactions*

Disease	OX-19	OX-2	OX-K
Spotted Fevers	4+ (to 1+)	1+ (to 4+)	—
Rickettsialpox	—	—	—
Epidemic Typhus	4+	1+	—
Brill-Zinsser Disease	variable	variable	—
Murine Typhus	4+	1+	—
Scrub Typhus	—	—	4+
Q Fever	—	—	—
Trench Fever	—	—	—

Agglutinins against Proteus strains OX-19 and OX-2, and rarely OXK, appear in the serum of patients with spotted fever. Agglutinins appear between the 5th and 12th days and single convalescent titers of ≥1:320 may be considered diagnostically significant.

Complement-fixation (CF) tests may be performed using ether soluble, group specific antigens to differentiate spotted fever rickettsiae from typhus and Q fevers. Washed, type specific corpuscular antigens are used to distinguish among the various members of the spotted fever group. CF antibodies appear during the 2nd or 3rd week of illness. Other serologic techniques including immunofluorescence, agglutination, and mouse toxin neutralization may be used for serologic evidence of disease.

The host range of *R. rickettsii* is broad and numerous species of ticks, small mammals, and man are infected in nature. The male guinea pig is the animal most frequently used for laboratory studies. When inoculated intraperitoneally with infectious material, it develops fever >40 C and a marked scrotal reaction consisting of a hemorrhagic rash followed by necrosis and ulceration of the skin. Necrosis and sloughing of ears and footpads may also occur. With virulent strains death may ensue during the 6th to 8th day of fever. Rickettsiae may be readily demonstrated in the spleen and tunica vaginalis, which are the tissues of choice for passage material.

Spotted fever rickettsiae are parasites of ticks which are distributed in several organs including the ovaries and salivary glands. Infected ova give rise to infected progeny, and rickettsiae in saliva are transmitted to man by tick bite. Man is only accidentally infected when he intrudes into the tick environment, and is in no way necessary for survival of these rickettsiae. As rickettsiae are passed transovarially, ticks serve not only as vectors, but also as primary reservoirs of disease. There is substantial evidence for natural infection of several wild vertebrates with *R. rickettsii* (meadow mice, cottontail rabbits, squirrels, and chipmunks). Antibodies have been detected in a myriad of animal species.

Control of RMSF is primarily a function of avoidance of tick infested areas. In addition man has been infected by accidental exposure to aerosols generated within the laboratory. A vaccine of questionable efficacy is available for personnel at great risk of contracting disease.

[B.] *Tick-borne spotted fever rickettsiae of the Eastern Hemisphere* include organisms which cause mild to moderate, generally nonfatal illness. These agents are widely distributed geographically and share a group antigen with *R. rickettsii* but have distinct type-specific corpuscular antigens. *R. conorii* is the prototype and causes disease throughout Africa, India and parts of Europe and the Middle East adjacent to the Mediterranean, and the Black and Caspian Seas. Clinical observations include an initial lesion (eschar), found at the site of tick attachment, headache, fever for up to two weeks, and a generalized maculopapular erythematous rash usually involving palms and soles. Pathologic lesions generally consist of thrombosis of capillaries, small arteries and veins with the earliest changes in the vascular endothelium. Chloramphenicol and the tetracyclines are effective therapeutically.

CF antibodies appear during the 2nd week; however, Weil-Felix agglutinins to *Proteus* OX-19 and OX-2 frequently are found only in low titers. The species may be differentiated by cross immunity in guinea pigs, mouse toxin neutralization and cross CF tests. Following intraperitoneal inoculation with *R. conorii* and *R. sibirica*, guinea pigs develop fever, splenomegaly and testicular changes. While mice and guinea pigs are susceptible to *R. australis*, this organism does not produce a "toxic effect" in mice.

The primary animal host of *R. conorii* is the dog, with *Rhipicephalus sanguineus*, the brown dog tick, serving as the principal vector. *R. australis* appears to have a reservoir in marsupials and at least four species of ticks are capable of infecting man. Animal hosts of *R. sibirica* have not been elucidated. Control of these diseases involves avoidance of ticks. Effective vaccines for prevention are not commercially available.

[C.] *Rickettsialpox* was first recognized in New York in 1946 and has subsequently been described in many locations including the Soviet Union. The disease is a self-limited, febrile illness transmitted from mouse to man by blood sucking mites, *Allodermanyssus sanguineus*. Like spotted fever rickettsiae, *R. akari* multiplies in both nucleus and cytoplasm of host cells and the common soluble antigen reacts in CF tests with antibodies to the spotted fever group. The type specific particulate antigen and cross-immunity in guinea pigs serve to differentiate *R. akari* from other members of the spotted fever group. This rickettsia does not elicit Weil-Felix agglutinins and has no "toxic effect" in mice. *R. akari* grows well in the yolk sacs of embryonated eggs, causes a fatal disease in laboratory mice, and guinea pigs develop fever and mild scrotal swelling.

The house mouse is the natural mammalian reservoir for *R. akari*, and disease is usually seen in urban centers. The mite bite develops into a papule which forms an eschar which resembles a vaccinia inoculation. The illness has an abrupt onset with fever, headache, and a characteristic vesicular rash which resembles chickenpox. The course of disease lasts from 1 to 2 weeks and is not fatal. Chloramphenicol and the tetracyclines are markedly effective in treatment of patients with Rickettsialpox. Control measures involve rodent control and insecticides for mite disinfestation.

[3.] *Typhus Group*

This group includes two species pathogenic for man, *Rickettsia prowazekii*, the etiologic agent of epidemic typhus fever and Brill-Zinsser disease, and *R. typhi*, which causes murine (endemic) typhus.

[A.] Epidemic Typhus Fever has been intimately associated with human misery, poverty, and overcrowding for centuries and is especially prevalent during warfare. It is reported to have helped destroy half a million men of Napo-

leon's Army of 1812; ravaged an estimated 30 million Russians during World War I; and during World War II it was heavily encountered in the European theater of operations as well as in Africa, the Far East, Japan, and Korea. Suffice it to say that typhus fever has been one of the major epidemic diseases in the history of man.* Epidemic typhus is characterized by high fever, severe headache and rash. During epidemics the case fatality rate is approximately 10% in persons between 20 and 30, and may rise to 60% in those over 50. The etiologic agent *R. prowazekii* was named by da Rocha-Lima in 1916 in honor of Dr. Howard Ricketts (American) and Dr. Stanislaus von Prowazek (Austrian) both of whom died of the disease while investigating its etiology.

In nature *R. prowazekii* infects only man and the human body louse, *Pediculus humanus* var. *corporis,* or less frequently the head louse *P. humanus* var. *capitis.* Body lice nest and lay eggs in clothing, and as the nymphs mature they emerge 4 to 6 times per day to take blood meals from the human host. If the ingested blood contains rickettsiae the louse becomes infected and organisms multiply in endothelial cells of the intestinal tract. These cells ultimately rupture, discharging rickettsiae into the feces. While feeding on human skin, the louse defecates, and since its bite causes itching, the host rubs the infected feces into broken skin. *R. prowazekii* are present only in the intestinal cells and feces of infected lice; they have not been demonstrated in other tissues and are not passed transovarially. Infected lice die within 1 to 3 weeks; therefore, the cycle of louse infection is required to maintain the human reservoir. Lice prefer a temperature approaching that of human skin, and, therefore, leave patients who either have high temperatures or who have died. Lice neither fly nor jump, and only crawl short distances which explains why epidemic typhus flourishes under conditions of extreme crowding. While monkeys, guinea pigs, rats, chick embryos and certain arthropods are susceptible, in varying degrees, to experimental infection with *R. prowazekii,* no natural reservoir other than man has been demonstrated.

Typhus rickettsiae are highly pleomorphic microbes 0.3 to 0.6 by 0.8 to 4.0 μm which occur singly, in pairs, or short chains. Optimal growth occurs in the yolk sac membrane of chick embryos and in a number of cell cultures. These rickettsiae replicate only in the cytoplasm of infected cells.

In man after a 10 to 14-day incubation period, the manifestations of epidemic typhus include abrupt onset with chills, fever, generalized pain, exhaustion, and headache (a hallmark of this disease). A generalized eruption usually appears on the trunk from the 4th to 7th day of disease, which spreads peripherally to the extremities (sparing face, palms, and soles). The macular (mulberry) rash may ultimately become petechial or frankly hemorrhagic. In severe cases prostration, stupor, delerium, renal insufficiency, and rarely gangrene of the extremities may occur. Thrombosis results from injury caused by rickettsial growth and rickettsiae may be demonstrated in endothelial cells. Necrotic areas of the skin appear to be associated with thrombosis of capillaries. In recovery the fever subsides during the 3rd week by rapid lysis. Pathological manifestations of disease can, in large

* For excellent background information the reader is referred to Zinsser's classic work: "Rats, Lice and History."

part, be accounted for on the basis of occlusion of small capillaries by swollen endothelial cells, thrombi, perivascular hemorrhages, extravasation of plasma, hemoconcentration and shock.

With tetracycline or chloramphenicol therapy patients usually respond within 12 to 24 hours and marked improvement occurs within 2 to 3 days. Recovery is accompanied by the absence of serious sequelae in addition to an enduring immunity to the disease.

Clinically, the early stages of typhus fever might be confused with smallpox, meningitis, measles, malaria, typhoid fever, and other infectious diseases. With the appearance of rash a more accurate diagnosis can be made. The erythematous rash of typhus spreads from trunk to extremities while the rash of RMSF ordinarily spreads centripetally. Epidemic typhus must then be differentiated from endemic typhus by means of serologic tests.

Laboratory diagnosis, routinely performed on the basis of serologic tests, detects antibodies by the Weil-Felix, complement-fixation, rickettsial agglutination, precipitin, opsonization, neutralization, or protection tests. Weil-Felix agglutinins (Table 2) for *Proteus* strain OX-19 may appear in the serum as early as the 7th or 8th day of illness, peak 2 to 3 weeks into the disease, then decline over a period of several months. Reliable results demand a titer of 1:160 or greater, on single serum specimens, or a fourfold rise in titer with paired sera. Agglutinins for OXK do not appear. From serologic studies it is evident that subclinical infection occurs, especially in children.

Male guinea pigs respond to epidemic typhus rickettsiae with fevers >40 C; and rarely, scrotal swelling. Guinea pigs do not develop a skin reaction and animals that survive do so without sequelae. Spleen or brain is the organ of choice for high titer passage material. Cotton rats are susceptible to lethal infection (with large doses of rickettsiae), whereas white rats are not; white mice undergo only an inapparent infection.

Control of epidemic typhus in man is predicated on vaccination or delousing of humans. The vaccine currently in use contains yolk sac grown rickettsiae which have been formalin killed and extracted with ether as described by Cox (1941) and Craigie (1945). This vaccine is reported to essentially eliminate mortality, and the course of disease in vaccinated individuals is milder and shorter than in naive populations. A second type of vaccine prepared from the living, attenuated strain E variant of *R. prowazekii* produces a long lasting immunity in man and is currently being tested in field trials.

Delousing of human populations has been used to control epidemics since the early 1900s. Once deloused with DDT, typhus patients are incapable of transmitting infection to others by contact, as rickettsiae are not found in saliva, sputum, urine or feces. Recent reports indicate, however, that human body lice are capable of developing resistance to DDT.

[B.] *Brill-Zinsser Disease* is a recrudescent form of typhus. It was first reported by Brill (1898) in New York as a sporadic form of typhus which occurred in immigrants from Eastern Europe. It differs from classic epidemic typhus in that (1) the disease is much milder, (2) lice need not be present, (3) rash is usually absent, and (4) antibodies ap-

pear very rapidly (3-4 days after onset). In 1934 Zinsser hypothesized that this disease was a recrudescence of epidemic typhus in persons who had previously experienced the disease, due to exacerbation of a latent infection with *R. prowazekii*. The rapid rise in antibody titer is associated with 7S globulin which is typical of an anamnestic response to an infectious agent. The Weil-Felix reaction is often negative in this disease; however agglutination and CF tests are normally positive.

The factors which precipitate recrudescence are unclear. Rickettsia apparently remain viable in sequestered sites in the body, even in the presence of circulating antibody. Disease is apparently precipitated when the delicate balance between the immune system and the parasite is disturbed.

The epidemiologic significance of Brill-Zinsser disease lies in the fact that carriers with latent infection may constitute a reservoir for disease. Given conditions of overcrowding and louse infestation, individuals with Brill-Zinsser disease are rickettsemic, so that they can serve as sources of infection for lice. Small outbreaks of louse-borne epidemic typhus have been described in Yugoslavia (1958) which originated from cases of Brill-Zinsser disease. These outbreaks were small due to the absence of crowding and low rates of louse infestation. There are no effective control measures for reducing the incidence of Brill-Zinsser disease. Chloramphenicol or tetracycline are used even though the disease is relatively mild and of short duration.

[C.] *Murine (Endemic) Typhus* is a relatively mild form of typhus fever transmitted from rats to man by the rat flea *Xenopsylla cheopis*. *Rickettsia typhi* causes a latent infection in the rat host and arthropod vector. The flea becomes infected by feeding on rats in the acute stage of disease; rickettsiae multiply in the epithelial cells of the intestinal tract; thereafter, rickettsiae are discharged in feces for the duration of the flea's life. Humans accidentally exposed to infected fleas are infected in a manner analogous to that described for epidemic typhus. The resultant disease is less severe than that caused by *R. prowazekii*, but the two are clinically indistinguishable. Case fatality rate is ca. 5% without antibiotic therapy and far less with chloramphenicol or tetracyclines.

No fundamental morphologic or biochemical differences exist between *R. typhi* and the rickettsiae of epidemic typhus. *R. typhi* and *R. prowazekii* are antigenically related, in that recovery from either infection leads to immunity to both diseases (due to a heat stable, common soluble antigen); whereas, immunization with killed organisms induces only homologous protection.

Differential diagnosis of endemic typhus is complicated as most reactions are analogous to those seen with epidemic typhus. Using specific, washed antigens, however, predominating antibodies are directed against *R. typhi* rather than *R. prowazekii*. In guinea pigs rickettsiae of epidemic typhus produce a mild disease, detected primarily by a rise in body temperature, while endemic typhus rickettsiae produce severe testicular lesions and marked swelling of the scrotum ("tunica reaction"). Endemic typhus rickettsiae may be maintained indefinitely by passage in white rats.

Endemic typhus is distributed worldwide, most frequently in locales where rats are present, and in this country is most prevalent in the southeastern states. Persons of all ages are susceptible. The use of DDT combined with rat poisons is effective in reducing the incidence of this disease.

[4.] *Scrub Typhus*

Scrub typhus, a disease caused by *Rickettsia tsutsugamushi*, was originally described in Japan and is currently recognized in a large triangular area roughly bordered by Japan, India and Australia. During World War II about 18,000 cases were reported among allied military personnel. Case fatality rates range from 1 to 35%, probably reflecting differences in virulence among diverse strains of *R. tsutsugamushi*. Scrub typhus is a zoonosis with rodents serving as vertebrate hosts and larval trombiculid mites (chiggers) acting as vectors. Over 400 species of *Leptotrombidium* mites have been described, but only 14 attack man. The species *L. akamushi* and *L. deliensis* are the most important vectors due to their widespread distribution. In a silent cycle the disease is one of rodents and is spread among them by mites; however, by intrusion man becomes accidentally infected. *R. tsutsugamushi* is not lethal for mites and is passaged transovarially; therefore, the mite may well serve as host and vector. Scrub typhus is contracted within "typhus islands" where rodents, mites and man come together. Typically this terrain is a cleared part of a forest, with low-lying grassy vegetation situated close to rice fields which attract rodents. The rodents become parasitized by mites, which then attack man. Alternatively, infections may be contracted in tropical rain forests. High humidity is a prerequisite for the maintenance of the characteristic focal areas of mite infestation.

When man is infected, multiplication of rickettsiae takes place at the site of inoculation often with the formation of a cutaneous eschar. Rickettsiae are disseminated and rickettsemia ensues. The characteristic pathologic lesions are found in the small blood vessels of the skin, heart, lungs and brain. Interstitial pneumonitis occurs in practically all fatal cases. The onset of illness is sudden, characterized by fever, chills, conjunctival injection, generalized lymphadenopathy, and severe headache. By the end of the first week a maculopapular rash appears on the trunk and spreads to the extremities; normally the hands and face are not affected. Fever increases during the first week and may reach 104°F; a cough may be present at this time. Toward the end of the 2nd week the rash fades, fever falls by lysis, the eschar is virtually healed and patients generally recover, occasionally with sequelae. Death, if it occurs, is normally due to secondary bacterial pneumonia, encephalitis, or circulatory failure. The illness may relapse, especially in cases where antibiotic therapy is begun too soon or the course is too brief. Since broad-spectrum antibiotics are rickettsiostatic in action, total recovery depends upon a suppression of rickettsial growth until the patient develops protective immunity. The response following administration of antibiotics is more rapid in scrub typhus than in other rickettsial diseases. Hence, if antibodies do not develop, the patient is susceptible to reinfection or recrudescence. Occasionally patients relapse and require a second course of antibiotic therapy (especially in those treated early). Significantly, recovered individuals are fully susceptible to infection with heterologous strains. Prevention of scrub typhus is predicated on control of the mite by thorough clearing of land then spraying with miticides such as dieldrin or lindane.

R. tsutsugamushi generally appears as a diplococcus or short rod, 0.4 by 0.8 to 2.0 μm, which may appear bipolar. It is relatively labile; has a slow rate of replication in the cytoplasm of infected cells; and induces interferon production *in vitro.* Unlike the typhus and spotted fever group organisms, these rickettsiae do not demonstrate intragroup reactivity but are unique by virtue of their antigenic heterogeneity. Representative prototypes include the Karp (New Guinea), Gilliam (Malaya) and Kato (Japan) strains. In addition, 2 other immunotypes, the Fan and Chon strains were identified in Thailand; other antigenically distinct strains probably exist. The full diversity among scrub typhus rickettsiae is not known. This strain specific reactivity accounts for the limited application of the CF test for diagnostic purposes. As previously indicated, individuals infected by one strain remain susceptible to infection with other strains, and due to this diversity, a satisfactory vaccine has not been prepared for complete protection against scrub typhus. The Weil-Felix response in scrub typhus is to the *Proteus* OX-K (Kinsbury) strain (Table 2). Agglutinins are found by the end of the 2nd week. No OX-2 or OX-19 antibodies develop. Although the Weil-Felix OX-K reaction is often positive, it may be negative even in persons from whom *R. tsutsugamushi* is isolated. Serologic diagnosis is based on a 4-fold or greater rise in OX-K agglutinins. While the CF test is not generally employed, indirect immunofluorescence and neutralization tests are specific and reliable for identification. Serologic studies indicate that there is a substantial incidence of subclinical infection, especially in endemic foci.

The host range of *R. tsutsugamushi* is broad. The animal of choice for laboratory studies is the white mouse. Seven to eight days post-infection ruffling of fur is observed, the abdomen swells, edema occurs and death ensues at about 14 days. Rickettsiae can be found in any affected tissue by impression smears, but the spleen is the organ of choice for passage material. Strains of *R. tsutsugamushi* vary widely in their virulence for mice; identification of strains may be performed by cross neutralization tests in mice. Cotton and white rats are also susceptible to infection. Chick embryos are normally killed six to ten days after inoculation, without pathognomonic lesions. The "toxic effect" for mice has been demonstrated only with the Gilliam strain.

[5.] Q Fever

Q fever, caused by *Coxiella burnetii,* is an acute systemic disease first described by Derrick (1937) in abbatoir workers in Brisbane, Australia. The causative agent was simultaenously isolated in Montana by Davis and Cox (1938) and has subsequently been shown to have a worldwide distribution. This organism was encountered on a large scale by American military personnel during World War II in the Mediterranean area ("Balkan grippe") and has been implicated as the causative agent in several epidemic outbreaks.

C. burnetii differs from other rickettsiae in several respects: (1) it does not *require* an arthropod vector to maintain itself successfully in nature and is generally transmitted to man by aerosol, (2) it is unusually stable outside of host cells, (3) disease in man is usually characterized by pneumonitis without rash, (4) it does not elicit agglutinins for *Proteus* OX strains (Weil-Felix reaction), and (5) it does not elicit a "toxic" reaction in experimental animals. In addition,

it does not share antigens with other rickettsiae, and *Coxiella* immune animals are not resistant to other rickettsiae.

C. burnetii is a highly pleomorphic intracellular parasite, typically occurring as a small bipolar rod 0.3 by 0.8 μm, but also appearing as a diplobacillus, a lanceolate rod or a coccus. It replicates preferentially in vacuoles of the host cell rather than in cytoplasm or nucleus. It is susceptible to digestion by lysozyme but not by trypsin, highly resistant to the adverse effects of chemical and physical agents, and can survive for inordinately long periods in the dried or frozen states. *C. burnetii* grows in the cytoplasm but not the nucleus of infected cells. It grows very well in yolk sacs of chick embryos and in a variety of cell cultures. The host range of *C. burnetii* is extremely broad. In nature, it infects at least 40 species of ticks, other arthropods, birds, bats, and many species of mammals, notably ungulates, rodents, and marsupials. Experimentally, mice, hamsters, and guinea pigs are susceptible to infection with *C. burnetii.*

The phenomenon of "phase variation" was described by Stoker and Fiset in 1956. Freshly isolated strains of *C. burnetii* are characteristically in phase I; however, upon adaptation to chick embryo yolk sacs in the laboratory they are converted to phase II. Any phase II strain may be converted back to phase I by a single passage in a susceptible animal (e.g., guinea pig). Laboratory animals respond to either phase of infecting *C. burnetii* by first producing phase II antibodies, then at about 30 days produce antibodies which react with both phase I and phase II. Humans rarely produce phase I antibodies. Phase I and phase II rickettsiae can be differentiated by buoyant density. In addition, the phase I antigenic component can be extracted from *C. burnetii* with trichloroacetic acid leaving particulate rickettsiae in phase II. It appears probable that the phase I antigen is superficially situated in the cell wall and is associated with pathogenicity while phase II antigen may be more deeply seated.

Q fever is rarely a fatal disease. The incubation period ranges from 10 to 17 days, and the onset is sudden with fever (101 to 104°F), chills, malaise, anorexia, myalgia and a characteristic severe frontal headache. The degree of pulmonary involvement varies considerably and patients may develop pneumonitis; however, Q fever is generally a systemic disease accompanied by rickettsemia. Recovery usually takes place rapidly without complications; although, severe weight loss, hepatic involvement, abortion or congenital malformation can be observed. Rickettsiae may persist in tissues and chronic Q fever may induce subacute endocarditis with concomitant microcolonies of *C. burnetii* in heart valve tissue. This form of disease is invariably fatal.

A diagnosis of Q fever in man is established by isolation of the organism from blood, sputum or lung, or demonstration of antibodies in serum. The infection is best confirmed in guinea pigs or embyronated eggs. Guinea pigs inoculated intraperitoneally develop temperatures >40°C after a variable prodromal period. Passage material is usually blood or spleen suspension. These animals develop specific antibodies within 14 to 30 days. Approximately 50% of chick embryos inoculated with *C. burnetii* die within 7 to 11 days.

Antibodies against *C. burnetii* are quite specific and a rise in titer between paired serum samples is evidence of current

disease. It should be noted that serologic studies suggest that in many instances infection is inapparent. The most widely used diagnostic techniques involve agglutination and direct CF. The microagglutination test with stained antigen appears to be more specific and more sensitive than the CF. In addition, agglutinins appear earlier (1st to 2nd week) than CF antibodies (2nd to 4th week). Corpuscular phase I antigens are used in the CF and agglutination tests; however, phase II antigens are used in the CF test only, since they tend to agglutinate spontaneously and are therefore unsatisfactory for the agglutination technique. Other serologic procedures which have recently been introduced include radioisotope precipitation of antibody and identification by fluorescence microscopy.

Chloramphenicol and tetracyclines are the drugs of choice for Q fever; however, there is evidence of relapses and primary illness resistant to antibiotic therapy. Therefore, these drugs are not as effective in the treatment of Q fever as they are for other rickettsial diseases.

Q fever is primarily a zoonosis, which is typically an inapparent infection of domestic livestock (cattle, sheep, goats). Man is incidental to the maintenance of disease and is accidentally infected. While Q fever may be transmitted in nature by ticks, human disease is normally acquired by inhalation, and may be the result of occupational exposure. Overtly healthy animals can shed large numbers of rickettsiae in milk, urine, feces, and placentae. In addition, rickettsiae may reside in wool, leather, and straw. The infectious dose for man may be as small as one microorganism. While *C. burnetii* infects many species of animals and birds, it apparently causes frank disease only in man and guinea pigs. Control of human disease is predicated on the use of specific vaccines made from killed rickettsiae. Unfortunately, the available products have undesirable side effects eliciting reactions at the site of injection. A live, attenuated vaccine strain (M-44) has been developed in the Soviet Union; however, this product elicits mild disease and available evidence indicates that the organism may persist in tissues.

[6.] *Trench Fever*

This disease was first recognized during World War I when approximately one million cases were reported. The disease reappeared in small outbreaks in Japan and Poland during the 1920's and 1930's, then in epidemic form on the Russian front during World War II. Strains of the causative agent *Rochalimaea quintana* have recently been identified in Tunisia and Mexico. The causative organisms are coccoid or coccobacillary 0.2 to 0.5 μm wide and up to 1.5 μm in length. They are often seen in pairs and tend to exhibit bipolar staining. With the exception of rhesus monkeys, none of the usual laboratory animals are susceptible to disease. Attempts to grow *R. quintana* in the embryonated egg and cell cultures have usually failed.

The human body louse *Pediculus humanus* var. *corporis* transmits the disease to man. *R. quintana* grows within the lumen of the louse gut rather than within intestinal epithelial cells. This fact, together with the finding that the organisms may be cultivated on blood agar place them in a genus apart from other rickettsiae.

Once a louse has been infected it excretes infectious particles in feces for the remainder of its life. The louse does not die as a result of infection and rickettsiae are not passed to progeny via ova. Man is normally infected by rubbing louse feces into a bite wound.

After a highly variable incubation period, disease in man begins suddenly with violent headache, dizziness, generalized aches and pains in the long bones (e.g., shins and thighs) and retroorbital pain. A discrete roseolar rash appears with fever, but subsides after about 30 hours. Chills and fever tend to subside and recur in repeated cycles of 3 to 5 days' duration ("five-day fever" from Latin: quintus). The illness may persist for months or, rarely, in excess of one year. The disease is not normally fatal and rickettsemia can be terminated by use of tetracyclines. The illness is one which occurs during warfare, natural disaster, or other occasions when personal hygiene is poor and louse infestation occurs. The presently available insecticides coupled with antibiotic therapy make it unlikely that this disease will recur in serious epidemics.

The Weil-Felix test is negative in Trench fever (Table 2). However, specific CF antigens have been prepared from *R. quintana* cultivated on blood agar. A toxic effect is unknown. The standard method of laboratory confirmation has traditionally been to allow healthy lice to feed on a patient, then examine louse feces for *R. quintana* and inoculate monkeys.

[7.] *Serologic Diagnosis*

Unless equipped with adequate protective facilities diagnostic laboratories should not routinely attempt animal inoculations necessary for definitive identification, as the isolation of rickettsial organisms involves hazards to technical personnel. Serologic methods are simpler and safer than isolation procedures and are, therefore, the preferred diagnostic approach used by most laboratories. In general, a demonstration of a significant rise in antibody (fourfold increase) between acute serum collected early in the course of disease and convalescent serum, collected 10 to 21 days later is presumptive evidence of infection. Antibodies to the etiologic agent normally appear during the 2nd week of illness and peak during or after recovery. Serologic tests may be used to detect antibodies elicited to: (1) ether soluble, group antigens which have broad characteristics for each of the major rickettsial groups, and (2) type specific antigens, associated with the rickettsial cell which differentiate species and strains within species. The types of serologic tests which may be performed by a diagnostic laboratory will be described in the following paragraphs. For a comprehensive discussion of each the reader is referred to recent publications by Elisberg and Ormsbee.

[A.] The *Weil-Felix (WF)* test is based on the agglutination of *Proteus vulgaris* strains OX-2, OX-19, and OX-K by antibodies formed in response to infection with rickettsiae of the typhus and spotted fever groups. Castaneda (1945) found that the agglutination of *Proteus* bacilli by antirickettsial serum could be explained on the basis of an antigen common to both organisms. Antigens and standard antisera are commercially available and this test is capable of establishing a useful presumptive diagnosis. The procedure is available as both a tube and slide test. Unfortunately, the reaction is nonspecific; with this technique it is not possible to differentiate between epidemic typhus, murine typhus and RMSF. In addition, positive reactions have been obtained in patients with nonrickettsial diseases, while some patients

never produce *Proteus* agglutinins. These antibodies have a tendency to disappear in late convalescence and, therefore, the WF test cannot be used for epidemiologic studies of past infections in a given area. WF agglutinins may not appear during the first week of illness but are usually present by the 14th day. They may be delayed by vigorous early treatment with antibiotics. Since OX-19 agglutinins as high as 1:80 occasionally appear in healthy persons, the lowest titer considered significant in a single serum specimen is 1:160, while in paired sera a 4-fold rise is required. Reactions normally encountered in the WF test are presented in Table 2.

[B.] The *complement fixation (CF) test* is more specific than the Weil-Felix reaction for the detection and identification of rickettsial antibodies. However, the CF test can produce erroneous and misleading information unless details are rigorously and faithfully followed for reliable and reproducible results. Antibodies to both group and typespecific antigen may be detected by the CF test. This test is impractical for diagnosis of scrub typhus due to the multiplicity of strains. CF antigens are commercially available for phase II Q fever, typhus and spotted fever groups.

[C.] *Rickettsial agglutination tests* with highly purified antigens are more species-specific than the CF test. The test may be performed by either macro (tube) or micro (slide or Microtiter) methods. The microagglutination (MA) test described by Fiset, *et al.* (1969) is highly sensitive in detecting antibody; the good reproducibility of results is a strong recommendation for its general use when appropriate antigens are available. The major drawback of the test is the lack of commercially available antigens normally prepared from embryo cells or infected yolk sac tissues. In addition, phase II *C. burnetii* cannot be used in the agglutination test because of their tendency to aggregate spontaneously. In the MA test formalin killed, purified rickettsial antigen suspensions are standardized to contain approximately 1 mg of rickettsiae/ml. Antigen and antibody in microtiter plates are incubated at room temperature for 18 hours after which time 25 μl of 0.02% (W/V) acridine orange is added. Agglutination results are recorded at 24 hours. It has been reported that rickettsial agglutinins appear earlier and persist longer than CF antibodies. The capillary agglutination test, employing hematoxylin stained *C. burnetii*, recently was developed as a seroepidemiologic tool to detect Q fever antibody in serum, milk, or other opaque fluids.

[D.] *Immunofluorescence (IF) methods* have been used for the detection of rickettsial antigens in cells and for demonstration of rickettsial antibodies in serum. The direct technique using fluorescein-labeled antibody has been most successfully used for the observation of rickettsiae in arthropod vectors (e.g., hemolymph), infected tissues and cell cultures. The indirect method involves the initial reaction of specific antibody with antigen; this complex is then reacted with fluorescein-conjugated secondary antiserum against the specific species of antibody used in the test (e.g., antihuman). This is the technique generally used for the detection of antibodies in serum specimens, and is the most reliable test available for the diagnosis of scrub typhus. In addition, the test can distinguish between sera from murine and epidemic typhus patients. The direct IF technique is simple and relatively inexpensive but requires quantities of high-

titer specific antiserum, while the indirect IF technique is not quite as simple, but is more flexible. Unfortunately, slide preparations of antigen (preferably infected tissues) are not commercially available; however, slides prepared in the laboratory can be fixed in acetone and preserved at −70°C indefinitely. Recently, a microimmunofluorescence technique has been described which is both practical and reliable. The test is more sensitive than the CF test and detects antibodies as early as 5 to 7 days after onset of illness and apparently after CF antibodies have disappeared. Briefly, slides containing 25-50 μl "spots" of fixed antigen are flooded with antiserum and then examined by the indirect fluorescent antibody (IFA) method. Using human or primate antisera we have found excellent correlation between the IFA and MA tests.

[E.] Chang, *et al.* (1953, 1954) described a *hemagglutination technique* in which alkaline extracts of typhus and spotted fever group rickettsiae could be used to sensitize human or sheep erythrocytes which could then be agglutinated by convalescent human sera. The group specific erythrocyte sensitizing substance (ESS) is stable and contains proteins and carbohydrate. It may be distinct from the CF antigen, rickettsial agglutinogen and *Proteus* common antigen. Shirai, *et al* (1975) have recently described an indirect hemagglutination (IHA) test using gluteraldehyde-stabilized sheep erythrocytes treated with group-specific ESS obtained from *R. rickettsii*. While this technique is apparently not as sensitive as IFA, the method is simpler than CF and results compare favorably with CF and Weil-Felix tests.

[F.] In 1911 Ricketts reported that spotted fever immunity could be passively transferred by convalescent serum to normal animals. According to Parker's *protection or neutralization* test, a serum sample from a patient is added to blood taken from infected guinea pigs, and then injected intraperitoneally into normal guinea pigs. If protective antibodies are present in the serum of the patient, guinea pigs will be protected from disease. The technique is expensive in terms of animals, laboratory space, and time, and can be hazardous.

[G.] In the *"toxin" neutralization test* the "toxic" effect of the typhus and spotted fever groups can be abated by mixing rickettsial suspensions with protective antiserum prior to injection. This test is still used for the standard assay of epidemic typhus vaccine. Neutralization of either toxicity or infectivity depends on antibodies to the type specific antigen.

[H.] In addition, *in vitro* measurements for neutralizing antibody may be performed using tissue culture. Convalescent serum will bind to, but not neutralize, spotted fever rickettsiae. However, infectious complexes are neutralized by the addition of anti-immunoglobulin and subsequent incubation. This *indirect plaque reduction technique* is not generally recommended for the routine clinical laboratory detection of rickettsial antibody.

[I.] While a *radioisotope precipitation (RIP) technique* has been described for Q fever serology, this procedure has not been adequately adapted to diagnosis of other rickettsial diseases. Basically, antigen labeled with isotope is sensitized with suspect serum, then incubated with appropriate secondary antiglobulin. This suspension is differentially centrifuged, the precipitate and the supernatant counted, and

the radioactivity in both determined. The amount of isotope precipitated is an indication of the antibody activity.

[J.] Serum opsonizing activity has been studied using a *radiometabolic assay*. Briefly, peripheral polymorphonuclear leukocytes (neutrophils) are separated from blood by dextran sedimentation. Serum, inactivated rickettsial antigen, balanced salt solution and [^{14}C] glucose are incubated in the presence of hyamine hydroxide. After opsonization, leukocytes are added, released $^{14}CO_2$ is measured and opsonizing activity is equated to hexose monophosphate shunt activation in terms of $^{14}CO_2$ released from the oxidation of [^{14}C] glucose by the polymorphonuclear cells. Opsonizing antibodies have been demonstrated in infections with typhus and spotted fever group rickettsiae.

[K.] Assays of immunity by cell-mediated responses have only recently come under close scrutiny. Comparatively little information exists concerning the cell-associated immune response to rickettsial diseases. Coonrod and Shepard have examined the *lymphocyte transformation* response of human peripheral blood leukocytes to rickettsial antigen. Using a modification of the method of Bach and Hirschhorn (1964) [^3H] thymidine uptake was demonstrated in the stimulated lymphocytes of individuals previously affected with RMSF. *Inhibition of macrophage migration (MIF) has* also been demonstrated in animals previously infected with *R. rickettsii*. The technique of Harrington and Stastny has been in the immobilization of the movement of polymorphonuclear cells. Lymphocyte transformation has the demonstrated advantage that several data points may be obtained from the same animal, while in contrast, animals must generally be killed to obtain peritoneal macrophages for use in the MIF test.

Recommended Supplemental Reading

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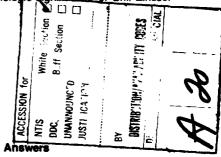5. Joklik, W. K., and Smith, D. T. *Zinsser Microbiology,* Fifteenth edition. Appleton-Century-Crofts, New York, 1972, pp. 671-684.
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12. Zinsser, H. *Rats, Lice and History.* Little, Brown and Co., Boston, 1935.

Self-Assessment Quiz

Questions

1. What are the rickettsial diseases endemic to North America, and what are the respective etiologic agents?
2. Describe those characteristics which lead to the classification of rickettsiae as bacteria.
3. In what way do rickettsia resemble viruses?
4. What are the antibiotics of choice for the treatment of rickettsial diseases?
5. How are species of the genus *Rickettsia* primarily transmitted?
6. How is *Coxiella burnetii* normally transmitted?
7. Species of Rickettsia grow only in actively growing host tissue such as laboratory _____, embryonic _____, or tissue _____.
8. List five serologic techniques which may be used as indicators of rickettsial disease.
9. Rocky Mountain spotted fever rickettsiae are transmitted to man by the bite of infected _____, Rickettsial pox rickettsiae by _____, epidemic typhus rickettsiae by _____, and endemic typhus rickettsiae by _____.
10. What is the epidemiologic significance of Brill Zinsser disease?

Answers

1. The rickettsial diseases endemic to North America include: Rocky Mountain spotted fever *(Rickettsia rickettsii),* Rickettsial pox *(R. akari),* Brill Zinsser disease *(R. prowazekii)* endemic typhus *(R. typhi)* and Q fever *(Coxiella burnetii).*
2. Both have similar morphology, contain DNA and RNA, contain muramic acid and diaminopimelic acid in cell walls, possess metabolic enzymes, divide by binary fission and growth is inhibited by antibacterial compounds.
3. Both are obligate intracellular parasites.
4. Broad spectrum tetracyclines and chloramphenicol.
5. Species of *Rickettsia* are transmitted from arthropod to arthropod via infected ova; mammal to arthropod via infected blood; arthropod to mammal via direct inoculation.
6. *Coxiella burnetii* (Q fever) is normally transmitted via infectious aerosols.
7. Animals, eggs, culture.
8. Weil Felix test, complement fixation, agglutination, immunofluorescence, hemagglutination, neutralization, precipitation, radiometabolic assay.
9. Ticks, mites, lice, fleas.
10. Human carriers with a latent infection may constitute a reservoir for overt disease outbreaks.